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Physiological and biochemical appraisal for mulching and partial rhizosphere drying of cotton

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Abstract: Water is the main factor for the healthy life of plant. One of the main negative effects of climate change is the increasing scarcity of water that is lethal for plant. Globally, for water deficit regions (arid and semi-arid), drought is the main factor responsible for low production of agriculture, especially for cotton. Great efforts have been and are being made to find alternatives to water saving practices. This study aimed to examine the effects of partial rhizosphere drying (PRD, half of the root system irrigated at one event, and the other half irrigated in the next event, and so on) with and/or without various mulching treatments on physiological and biochemical traits of cotton. To explore this objective, we laid out experiments in completely randomized design with factorial arrangement in the Islamia University of Bahawalpur, Pakistan in 2016. Two factors included were four mulching treatments (M_0, no mulching; M_1, black plastic mulching; M_2, wheat straw mulching; and M_3, cotton sticks mulching) and two irrigation levels (I_0, control (full irrigation); and I_1, PRD). Fisher's analysis of variance among means of treatments was compared using least significant difference test at 5% probability level. Results revealed that the maximum plant height, leaf area, leaf gas exchange (photosynthetic rate and stomata conductance), chlorophyll, proline and total sugar contents, and enzyme activities were higher under M_2 than under other three mulching treatments. As for irrigation levels, higher values of plant height, photosynthesis and water related parameters (leaf water potential, leaf osmotic potential, leaf turgor potential, etc.) were recorded. Contents of total sugar and proline and activities of antioxidant enzymes were significantly higher in PRD-treated plants than in control plants. It was concluded that combined application of PRD and mulching was more effective than the rest of the treatments used in the experiment. Similar study can be conducted in the field by applying irrigation water in alternate rows in semi-arid regions.

Keywords: antioxidant enzymes; mulching; partial rhizosphere drying; photosynthetic rate; stomatal conductance

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1 Introduction

Cotton is a major cash crop that is used for natural fiber cloth production in many countries in the world (Patil et al., 2011). Pakistan ranks the fourth in cotton production, after China, USA and India (Gop, 2015–2016), and cotton contributes 7.1% in value added and 1.5% in its gross

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domestic product. Cotton is mainly grown in areas having low availability of water and is always artificially irrigated (Tang et al., 2005). There is, however, a need for innovative and efficient irrigation systems to compensate for water requirement of cotton and alleviate drought stress (Nasrullah et al., 2011). Different management techniques or conservation approaches to sustain drought are available, such as the application of growth regulators/nutrients (Raza et al., 2012a), usage of attuned osmolytes/solute/osmoprotectants (Raza et al., 2012b), management through bed planting and usage of different mulching materials or patterns (Ahmad et al., 2015).

Mulching is a water conservation technique practiced in arid and semi-arid regions to reduce water losses through runoff and evaporation (Ahmad et al., 2015). Moreover, activities of antioxidant enzymes in cotton were higher under mulching than under no mulching treatments (Meloni et al., 2003). Worldwide, black plastic mulching is the most commonly applied (Ngouajio and McGiffen, 2004; Ai et al., 2018). Wheat/paddy straw mulching can also be used and have been reported to improve the growth, development and yield of peanut and tomato compared to black plastic mulching or no mulching (Arin and Ankara, 2001; Ghosh et al., 2006).

Partial rhizosphere drying (PRD) is an efficient method of irrigating the root-zone alternatively to elevate the production of abscisic acid (ABA). The main principal is to wet half of the root portion in alteration of keeping the other half dry in each irrigation event during the crop growth season (Dry et al., 2000; Stoll et al., 2000). This has been proven to increase the biochemical signalings that are essential in lowering the stomata conductance with minimum or no effect on photosynthesis and productivity of the crop (Dry et al., 2000; Stoll et al., 2000). PRD has been profitably implemented in various crops (olive tree, maize, tomato, orange, grape, wheat and cotton) with water conservation up to 50% without causing significant reduction in final crop yield (Dry et al., 2000).

Maintenance of turgor and production of antioxidant enzyme activities are helpful in reducing the damaging causes of drought. Enzymes mostly involved in this defense mechanism are catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) (Raza et al., 2017). Previous studies revealed that activities of these enzymes significantly increased under PRD irrigation (Javanovic and Stikic, 2018), thus reducing the harmful effects of drought in cotton (Ullah et al., 2017). Similarly, mulching maintains the water balance (osmotic and turgor potentials) that is necessary for normal cotton growth (Saleem et al., 2016). As the application of PRD or mulching is favorable for counteracting drought so their combination should be investigated to estimate the extent of drought mitigation in cotton.

This study involved the sole and combined application of mulching and PRD irrigation on cotton grown in an arid region of Pakistan to confirm that either the sole use of these techniques or combined was more effective. The main aim was to pinpoint the most efficient irrigation technique and mulching materials for sustainable cotton production in arid regions.

2 Materials and methods

2.1 Study area and experimental design

The experiment was conducted at the experimental station of the Islamia University of Bahawalpur, Pakistan. The climate is continental and semi-arid. There was no precipitation and the average temperature was around 40°C during the study period in 2016.

The experiment was conducted from early June to early August 2016 as completely randomized factorial trial with four mulching treatments (M_0, no mulching; M_1, black plastic mulching; M_2, wheat straw mulching; and M_3, cotton sticks mulching) and two irrigation levels (I_0, control (full irrigation); and I_1, PRD, half of the root system irrigated at one event, and the other half irrigated in the next event, and so on). Totally 32 plastic pots (4 mulching treatments×2 irrigation levels×4 replicates), each with a 0.028 m^3 volume, were filled with 20-kg sieved dry sandy loam soil. The concentrations of readily available phosphorous (P), potassium (K) and nitrogen (N) in the soil were, respectively, 106, 90 and 12 mg/kg, pH was 7.6, organic matter content was 0.9%, pot capacity of soil (0–20 cm) was –0.068 Mpa (Baver et al., 1972) and the soil moisture (0–20 cm) at the time of sowing was 25%. Surface disinfected seeds of cotton MM-58, a semi-drought tolerant

variety, were obtained from the Regional Agricultural Research Institute in Bahawalpur, Pakistan, and sown on 1 June 2016 at a distance of 10 cm. All plants were removed from the pots 10 d after seedling emergence and their root system was settled in two plastic bags (Stikic et al., 2003). The aim was to divide the root system for application of the I_1 treatment. Vertical partition of polyethylene plastic sheet was used between each seedling line to control the water movement during each alternate irrigation event. Thereafter, the plants were equally irrigated for one week to properly establish the root system, after which period irrigation proceeded according to the treatment (I_0 or I_1). The pots were irrigated every three days until 50 d after repotting, when the experiment terminated. The total amount of water applied during the growth period was 120 mm in I_0 compared with 60 mm in I_1. The pots' surface was treated by mulching according to the treatments, i.e., M_0, M_1, M_2 and M_3.

2.2 Measurements and calculations

Plant height (m) was measured with a wooden meter rod. Leaf area index (LAI, m^2/m^2) was measured with portable laser leaf area meter (CI-2002L, CID Bio Science, United States). Leaf relative chlorophyll content (%) was measured by chlorophyll meter (CL-01, Hansatech instruments Ltd., United Kingdom) (Raza et al., 2017). To calculate the leaf relative water content (LRWC, %), we sampled the youngest fully expanded leaves and recorded their fresh weight (FW, g) and soaked the leaves for 24 h at 25°C in distilled water, after which turgid weight (TW, g) was recorded. Leaves dry weight (DW, g) was determined after oven drying at 70°C for 3 d. LRWC was calculated according to Equation 1 (Ahmad et al., 2015):

$$\mathrm{LRWC}(\%)=(\mathrm{FW}-\mathrm{DW})/(\mathrm{TW}-\mathrm{DW})\times 100\%. \tag{1}$$

Leaf water potential (MPa) was measured with water potential apparatus (Chas W. Cook, Birmingham, England). To compute leaf osmotic potential (MPa), we sampled the same leaves with measured water potential, i.e., frozen in liquid N and after 10 d the frozen sap was removed with special equipment for sap extraction and the sap was used for determining osmotic potential through a vapor pressure osmometer (Wescor 5520, Logan, USA). Leaf turgor potential (MPa) was calculated as the difference between water potential and osmotic potential (Rizwan et al., 2015).

Net photosynthesis rate (μmol/(m^2•s)) and stomatal conductance (mmol/(m^2•s)) of cotton leaves under natural conditions (Raza et al., 2017) were measured with, respectively, an infrared gas analyzer (Li-COR-LI 6250, Hertford, Herts, England) and an automatic porometer (MK-3, Delta-T Devices, Burwell Cambridge, England).

For the determination of protein content in the cotton leaves, bovine serum albumin was used as a standard (Bradford, 1976). The activities of SOD, POD, CAT and APX were measured according to, respectively, Giannopolitis and Ries (1977), Maehly and Chance (1954), Beers and Sizer (1952) and Anderson et al. (1992). Proline content was determined by the standard procedures of Bates et al. (1973) and total sugar content was analyzed by the method of Nelson (1944). Top soil (0–20 cm) moisture content was determined gravimetrically after oven drying.

2.3 Statistical analysis

The data for each variable was analyzed for detecting differences among the treatments' means. The analysis was conducted by the Fisher's analysis of variance and the least significant difference test at 5% probability level (Steel et al., 1997).

3 Results

All studied cotton parameters were significantly affected by single mulching and irrigation treatments (Table 1). The parameters were also affected by mulching and irrigation interactions, though slightly lower significance than that of single treatment effect, and some such as leaf chlorophyll and proline contents, APX and CAT activities and turgor potential were not affected by interaction of treatments.

Table 1 Summary of analysis of variance for the studied parameters of cotton under different mulching and irrigation treatments

Parameter	Mulching (M)	Irrigation (I)	M×I
Growth and photosynthesis parameters			
Plant height	0.00**	0.00**	0.040*
Leaf area index	0.00**	0.00**	0.004*
Relative leaf chlorophyll content	0.00**	0.00**	0.870ns
Stomatal conductance	0.00**	0.00**	0.002*
Photosynthetic rate	0.00**	0.00**	0.024*
Water related parameters			
Leaf relative water content	0.00**	0.00**	0.030*
Leaf water potential	0.00**	0.00**	0.000**
Leaf osmotic potential	0.00**	0.00**	0.040*
Leaf turgor potential	0.00**	0.00**	0.082ns
Soil moisture	0.00**	0.00**	0.000**
Biochemical parameters			
APX activity	0.00**	0.00**	0.844ns
CAT activity	0.00**	0.00**	0.138ns
POD activity	0.00**	0.00**	0.000**
SOD activity	0.00**	0.00**	0.000**
Total sugar content	0.00**	0.00**	0.022*
Proline content	0.00**	0.00**	0.051ns

Note: APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase. * and ** indicate significant differences at P<0.05 and P<0.01 levels, respectively. ns, non-significant.

3.1 Growth and photosynthesis parameters

Regarding the mulching effect, M_2 (wheat straw mulching) consistently yielded the highest values of growth and photosynthesis, closely followed by M_1 (black plastic mulching), while M_0 (no mulching) yielded the lowest values of growth and photosynthesis, and M_3 (cotton sticks mulching) was in-between (Table 2). The I_0 (full irrigation) yielded significantly higher values for growth and photosynthesis parameters than I_1 (PRD irrigation). In addition, interactions of all treatments involving I_0 yielded significantly higher values than the other combination treatments, and this was particularly evident for the interactions of M_1I_0 and M_2I_0. Relative leaf chlorophyll content was the only photosynthetic parameter without significant interaction effect.

3.2 Water related parameters

The treatments had also significant effects on the water related parameters (Table 3). For mulching, similar trend could be observed in the growth parameters, i.e., values were in the following order M_2>M_1>M_3>M_0, except leaf water potential that significantly yielded higher value for M_0 than those for M_1 and M_3 (Table 3). For irrigation, I_0 significantly yielded higher values than I_1 for all water related parameters. Among the interactions, the maximum LRWC (88.00%) was recorded in M_2I_0 and the minimum value was recorded in M_0I_1 (64.33%).

Soil moisture is also shown in Table 3 and mulching and irrigation interactions had a significant effect on soil moisture of cotton. The highest soil moisture (15.48%) was measured for M_1, followed by M_2 (12.63%) and the lowest soil moisture was measured for M_3 (10.75%). Regarding irrigation, higher soil moisture (17.45%) was observed in I_0 (control) than in I_1 (PRD, 6.62%). Among the interactions, the highest soil moisture (22.50%) was recorded in M_1I_0 and the lowest soil moisture was recorded in M_0I_1 (4.13%; Table 3).

Table 2 Effects of different mulching and irrigation treatments on growth and photosynthesis parameters of cotton

Treatment	Plant height (cm)	Leaf area index (m^2/m^2)	Relative leaf chlorophyll content (%)	Stomatal conductance (mmol/(m^2•s))	Net photosynthetic rate (μmol/(m^2•s))
Mulching (M)					
M_0 (no mulching)	21.83^d	0.78^d	34.06^d	312.83^d	13.50^c
M_1 (black plastic mulching)	33.67^b	1.11^b	39.75^b	338.33^b	15.28^b
M_2 (wheat straw mulching)	35.67^a	1.40^a	43.25^a	357.50^a	16.81^a
M_3 (cotton sticks mulching)	25.50^c	0.93^c	37.30^c	319.17^c	14.26^c
LSD	1.76	0.05	1.13	5.90	0.97
Irrigation (I)					
I_0 (control)	34.12^a	1.50^a	43.96^a	377.25^a	17.76^a
I_1 (PRD)	24.20^b	0.61^b	33.21^b	286.67^b	12.16^b
LSD	1.24	0.04	0.79	4.17	0.68
M×I					
M_0I_0	25.50^c	1.20^d	39.50	365.67^c	15.50^c
M_0I_1	28.16^e	0.36^h	28.60	260.00^g	11.50^e
M_1I_0	39.33^a	1.50^b	45.30	384.33^b	18.40^b
M_1I_1	28.00^b	0.73^f	34.20	292.33^e	12.16^{de}
M_2I_0	41.67^a	1.90^a	48.50	398.00^a	20.33^a
M_2I_1	29.67^b	0.90^e	38.00	317.00^d	13.30^d
M_3I_0	30.00^b	1.40^c	42.50	361.00^c	16.83^c
M_3I_1	21.00^d	0.46^g	32.10	277.33^f	11.70^e
LSD	2.49	0.07	NS	8.35	1.37

Note: Different lowercase letters within a column indicate significances among different mulching and irrigation treatments and their interactions at P<0.05 level. LSD, least significant difference; NS, non-significant.

Table 3 Effects of different mulching and irrigation treatments on water related parameters of cotton

Treatment	Leaf relative water content (LRWC; %)	Leaf water potential (MPa)	Leaf osmotic potential (MPa)	Leaf turgor potential (MPa)	Soil moisture (%)
Mulching (M)					
M_0 (no mulching)	71.67^d	–2.21^b	–12.65^d	–10.43^d	9.30^d
M_1 (black plastic mulching)	78.33^b	–2.09^c	–15.60^b	–13.50^b	15.48^a
M_2 (wheat straw mulching)	82.00^a	–2.34^a	–17.98^a	–15.64^a	12.63^b
M_3 (cotton sticks mulching)	74.50^c	–1.80^d	–14.00^c	–12.20^c	10.75^c
LSD	1.26	0.07	0.35	0.33	0.60
Irrigation (I)					
I_0 (control)	83.00^a	–1.72^b	–12.90^b	–11.17^b	17.45^a
I_1 (PRD)	70.25^b	–2.50^a	–17.21^a	–14.71^a	6.62^b
LSD	0.89	0.05	0.25	0.23	0.42
M×I					
M_0I_0	79.00^c	–1.73^f	–10.60^g	–8.86	14.46^d
M_0I_1	64.33^g	–2.70^b	–14.70^d	–12.00	4.13^g
M_1I_0	85.00^b	–1.81^{ef}	–13.50^e	–11.68	22.50^a
M_1I_1	71.67^e	–2.36^c	–17.70^b	–15.33	8.46^e
M_2I_0	88.00^a	–1.85^e	–15.50^c	–13.65	17.16^b
M_2I_1	76.00^d	–2.83^a	–20.46^a	–17.63	8.10^e
M_3I_0	80.00^c	–1.50^g	–12.00^f	–10.50	15.70^c
M_3I_1	69.00^f	–2.10^d	–16.00^c	–13.90	5.80^f
LSD	1.78	0.10	0.50	NS	0.85

Note: Different lowercase letters within a column indicate significances among different mulching, irrigation treatments and their interactions at P<0.05 level. LSD, least significant difference; NS, non-significant.

3.3 Biochemical parameters

Biochemical parameters for assessing effects of mulching and irrigation treatments on cotton involved total sugar and proline contents, SOD, POD, CAT and APX activities are presented in Figures 1–3. As shown in the figures, all parameters showed significantly higher amounts under I_1 than under I_0, and some parameters such as proline content, SOD and POD activities under I_1 were two times those of under I_0. In addition, mulching had a significant effect and all parameters responded in the following order M_2>M_1>M_3>M_0. Among the interactions, the treatments involving I_1 showed the highest values for all biochemical parameters, and this was particularly evident for M_2I_1.

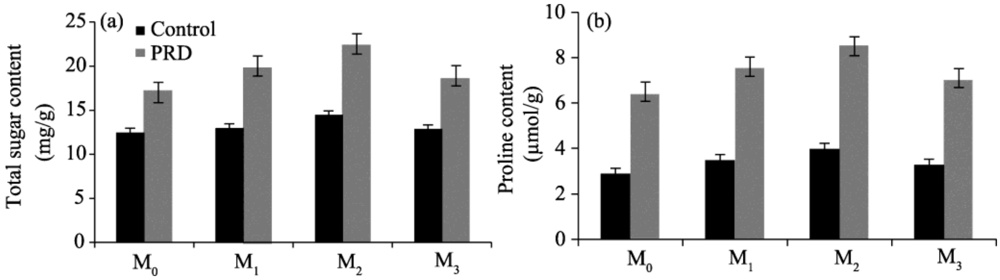

Fig. 1 Total sugar (a) and proline (b) contents in cotton under different mulching and irrigation treatments. M_0, no mulching; M_1, black plastic mulching; M_2, wheat straw mulching; M_3, cotton sticks mulching; Control, full irrigation; PRD, partial rhizosphere drying. Error bars indicate standard errors; *n*=4.

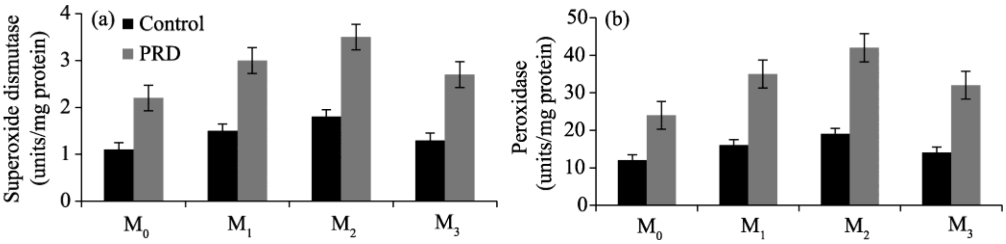

Fig. 2 Superoxide dismutase (SOD; a) and peroxidase (POD; b) activities in cotton under different mulching and irrigation treatments. M_0, no mulching; M_1, black plastic mulching; M_2, wheat straw mulching; M_3, cotton sticks mulching; Control, full irrigation; PRD, partial rhizosphere drying. Error bars indicate standard errors; *n*=4.

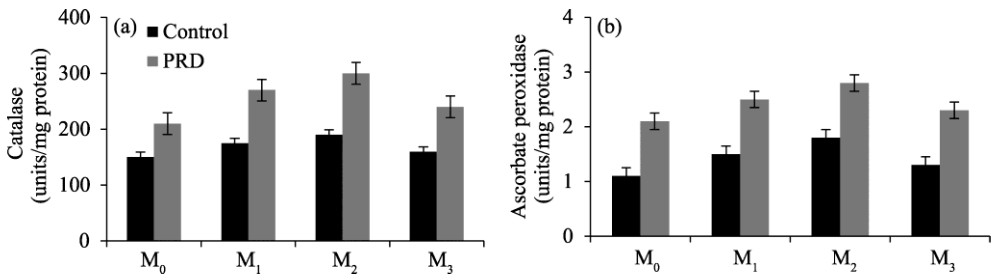

Fig. 3 Catalase (CAT; a) and ascorbate peroxidase (APX; b) activities in cotton under different mulching and irrigation treatments. M_0, no mulching; M_1, black plastic mulching; M_2, wheat straw mulching; M_3, cotton sticks mulching; Control, full irrigation; PRD, partial rhizosphere drying. Error bars indicate standard errors; *n*=4.

4 Discussion

4.1 PRD affecting the growth, photosynthesis and water relation of cotton

This study shows that mulching and PRD irrigation have positive influences on the growth of cotton. Generally, the cotton yield is largely dependent on its height as higher plant develops more nodes, branches and leaves that are the main sites for photosynthesis (Ahmad et al., 2015). A

lower plant height under PRD irrigation is probably due to less cell division and turgidity (Raza et al., 2017). Stikic et al. (2003) also recorded a lower plant height under PRD irrigation than under full irrigation. Plants had a higher height under mulching than under no mulching treatment due to more soil moisture conservation and minimization of evaporation losses (Ahmad et al., 2015). In addition, fully irrigated plants had a higher LAI than PRD-treated plants (Table 2) due to leaf area adjustment processes of plant under high soil water conditions (Ahmad et al., 2015). Among different mulching treatments, the maximum LAI was observed under M_2. Ahmad et al. (2015) also found a higher LAI in cotton under M_2 due to the higher soil water contents that result in more number and healthy growth of leaves (Hugar et al., 2009). Also, full irrigation resulted in the higher leaf chlorophyll content than PRD irrigation due to a lower efficiency of chlorophyllase (a major enzyme involved in chlorophyll formation) under drought condition (Saleh, 2012). High water conservation and availability to plants under mulching resulted in higher leaf chlorophyll contents than those of under no mulching. Similar results were reported by Hugar et al. (2009) and Nasrullah et al. (2011).

Chaves and Oliveira (2004) found that low photosynthetic efficiency with less water was mostly related to the stomatal closure, reduced conductance of mesophyll cells and ultimately less diffusion of carbon dioxide in the carboxylation sites of leaves. Moreover, a higher content of ABA under PRD irrigation further decreased the stomatal conductance, thus resulting in the low net photosynthesis rate of plant (Wang et al., 2005; Alkhaldi et al., 2012). However, researchers concluded that, although stomatal conductance decreased under PRD irrigation, there was no significant influence on the net photosynthesis rate in plants (Ahmadi et al., 2010; Baloch et al., 2012). In addition, the higher soil moisture availability under mulching resulted in the increase in stomatal conductance (Ahmad et al., 2015).

Raza et al. (2017) found that the changes in leaf osmotic potential resulted in a higher leaf water potential in PRD-treated plants than in fully irrigated plants. We found that PRD-treated plants accounted about 25% more leaf osmotic potential than fully irrigated plants. Moreover, PRD-treated plants had a higher negative leaf turgor potential due to the lower leaf water potential and LRWC (Table 3).

4.2 PRD affecting the biochemical traits of cotton

Researchers showed that the higher leaf turgor potential resulted in the accumulation of osmotic materials such as total sugar content (Ali and Radwan, 2008; Chutia and Borah, 2012; Saleem et al., 2016; Raza et al., 2017). We also found that the contents of total sugar and proline significantly increased under PRD irrigation (Figs. 1 and 2). Furthermore, ABA accumulation in PRD-treated plant is also a main factor of enhancing the soluble sugars contents (Parida et al., 2007; Khan et al., 2012). Total sugar content increased in plants under mulching and the maximum total sugar content was found in plants under M_2 and PRD irrigation (Fig. 1).

Proline is an important osmolyte in plants and involves in stabilization of various organelles and macromolecules (Sumithra et al., 2006). The breakdown of large protein molecules under drought stress resulted in the increase in proline contents (Izanloo et al., 2008). Moreover, ABA regulates the *P5CS* gene expression that further increases the synthesis of proline (Ashraf and Foolad, 2007; Chen et al., 2018). Our results found that combined application of PRD and M_2 resulted in the highest proline content than the other combination treatments, which is in line with the findings of Bozorov et al. (2018).

Overproduction of reactive oxygen species under abiotic stresses can lead to the destruction of cell membrane (Dias et al., 2011) by stimulating lipid peroxidation (Wang et al., 2009; Mutlu et al., 2011). An effective antioxidant system is therefore necessary for the survival of cell and its organelles. Antioxidant enzymes such as SOD, POD, CAT and APX are known for the scavenging of reactive oxygen species. These enzymes reduce negative impacts of drought by reducing lipid peroxidation, ionic leakage and the concentration of malondiadehyde (Raza et al., 2017). Elevated activities of antioxidant enzymes were noted in PRD-treated plants and the highest efficiency of

antioxidant enzymes was found under M_2. Similar result was also found in the oilseed rape (Gu et al., 2018).

5 Conclusions

A better growth and a higher photosynthesis capacity in cotton were observed under full irrigation than under PRD irrigation; however, the proline and total sugar contents, and activities of antioxidant enzymes were significantly higher in cotton plants under PRD irrigation than under full irrigation. On the other hand, mulching had positive impacts on soil moisture and hence significantly improved the efficiency of PRD irrigation. Among mulching treatments, M_2 performed the best under PRD irrigation. Therefore, combined application of PRD irrigation and M_2 in the field deserves further attention to optimize cotton production with less water in arid regions.

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